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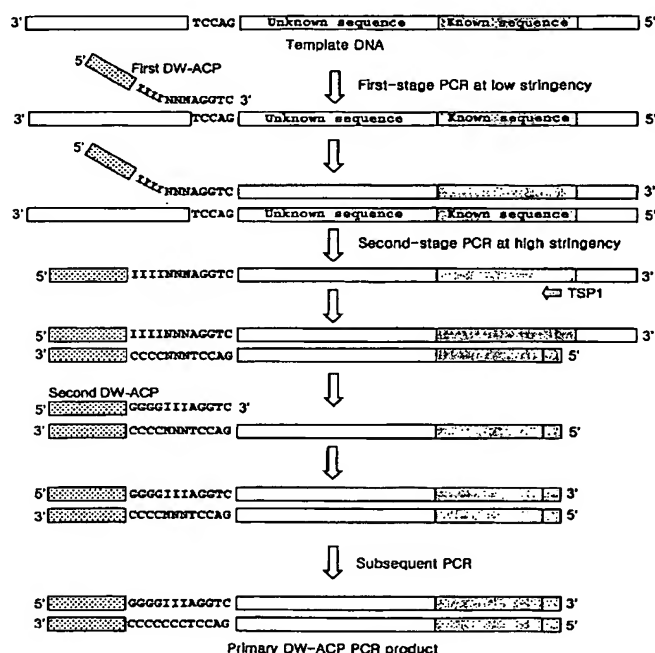
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(54) Title: METHOD FOR AMPLIFYING UNKNOWN DNA SEQUENCE ADJACENT TO KNOWN SEQUENCE



Known sequence  
Unknown sequence  
Universal sequence  
Target Specific Primer  
Deoxyinosine

(57) Abstract: The present invention relates to a method for amplifying an unknown nucleotide sequence adjacent to a known nucleotide sequence, which comprises the step of (a) performing a primary amplification of said unknown nucleotide sequence using a DNA walking annealing control primer (DW-ACP) and a first target-specific primer, in which said step (a) comprises: (a-1) performing a first-stage amplification of said unknown nucleotide sequence at a first annealing temperature, comprising at least one cycle of primer annealing, primer extending and denaturing using a first degenerate DW-ACP containing a degenerate random nucleotide sequence to hybridize with said unknown nucleotide sequence and a hybridizing nucleotide sequence substantially complementary to a site on said unknown nucleotide sequence; and (a-2) performing a second-stage amplification at a second annealing temperature to render said first degenerate DW-ACP not to function as a primer.

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